

Changes to the Specification

On page 1 before Field of Invention, please insert the following paragraph:

This application is a National Stage of International Application No. PCT/US99/07902, filed on April 8, 1999, under 35 U.S.C. §371, which claims the benefit under 35 U.S.C. 119(e) of U.S. Provisional Application 60/128,083, filed April 7, 1999, and U.S. Provisional Application 60/081,348, filed April 9, 1998.

On page 4, please amend the following paragraphs:

FIG. 1A shows the sequence of *Alfin1* cDNA (GenBank accession number L07291) (SEQ ID NO: 2).

FIG. 2 shows the DNA sequences that bind *Alfin1* *in vitro* (SEQ ID NOS: 3-11)

FIG. 3 shows the *MsPRP2* genomic region in *M. sativa* (Gen Bank accession number AF 028841) (SEQ ID NO:1).

On page 5, please amend the first paragraph:

...demonstrated. See FIG. 1, in which the *Alfin1* cDNA sequence (SEQ ID NO: 2) and deduced amino acid sequence are shown. Cys and His residues comprising the putative zinc finger are underlined. Dashed line indicates strongly acidic region of the protein. If *Alfin1* were to act as a transcription factor in root specific regulation, DNA binding of the protein might be expected. To test for sequence specific DNA binding, recombinant *Alfin1* protein was first expressed in *Escherichia coli* from the construct shown in FIG. 1B, in which the schematic representation of the pET-29b construct for *Alfin1* fusion protein is shown. The top line of the amino acid sequence shows the S-Tag and the biotinylated thrombin cleavage site of the vector. The *Alfin1* sequence below shows in bold the nine N-terminal amino acids deleted in the construct, the negatively charged region and the putative zinc binding domain with the relevant Cys₄, His/Cys₃ residues underlined. The affinity purified recombinant protein was shown to be authentic *Alfin1* protein by amino acid sequencing the amino terminal region of the protein. This sequence was identical to the sequence predicted from cloned cDNA as shown in Table 1 below.

On page 6, please amend the following paragraph:

...zinc finger protein to the EGR-1 consensus sequence. Science, 1990; 250, 1259-1262) and the bound DNA purified by four rounds of PCR amplification and binding, followed by cloning of the isolated sequences. Sequence analysis of the isolated clones (FIG. 2; SEQ ID NOS: 3-11) showed a consensus sequence in high affinity binding clones that was either GTGGNG or GNGGTG, confirming that *Alfin1* was indeed a specific DNA binding factor that could potentially function in gene regulation. See FIG. 2 in which (A) shows Consensus sequences aligned from individual clones that bind *Alfin1*, which were isolated after four rounds of gel retardation assays coupled with PCR amplification of the bound sequences and (B) shows sequence elements similar to those cloned by PCR amplification of *Alfin1* protein bound sequences that are found in the three *MsPRP2* promoter fragments which bind *Alfin1* protein in vitro.

On page 6 and continued on to page 7, please amend the following paragraph:

Alfin1 was found to show a strong root specificity in its expression pattern. Therefore, as a DNA binding protein it would be a likely regulator for root specific gene expression. Three fragments from the 1552 bp root specific and salt inducible *MsPRP2* promoter (FIG.3; SEQ ID NO: 1) from alfalfa (See: Bastola, Pethe and Winicov, 1998, supra) were found to bind recombinant *Alfin 1* protein *in vitro*, while a similar size control DNA fragment showed no DNA binding. See FIG. 3 in which DNA sequence of 1552bp of the *MsPRP2* promoter (SEQ ID NO: 1) is shown. (Underlined are: the translation start site at +1; the TATAA and CAAT sequences; the Tfil cleavage sites used for isolating Fragments 1, 2 and 3 for DNA binding experiments with recombinant *Alfin1*; the potential binding sites for *Alfin1* as well as myc and myb transcription factors as discussed in the specification. (*) indicates that the potential binding site is found on the complementary DNA strand. This nucleotide sequence data has been assigned accession number AF028841 by GenBank, an international genetic information data base operated by the United States of America. The binding to the *MsPRP2* promoter fragments was specific, could be inhibited by EDTA, was dependent on recombinant *Alfin1* protein concentration and showed different affinities for each individual fragment. The DNA sequence of each fragment contained a variant of the G rich consensus binding sequence for *Alfin1* protein that was identified in the

random oligonucleotide selection as shown in FIG. 2 (SEQ ID NOS: 3-11) and could account for the observed binding in gel retardation assays. The correlation of this finding with both *Alfin1* and *MsPRP2* expression in roots and *MsPRP2* inducibility by salt supported our hypothesis that *Alfin1* could play a role in gene expression and root maintenance in our salt-tolerant plants and suggested a potential role for *Alfin1* in strong root growth and development.

On page 15 and continued on page 16, please amend the following paragraphs:

FIG. 6C shows *Alfin1* and *MsPRP* expression in Roots and Leaves. Total RNA was isolated from roots and leaves of the same plant. #1 is control salt-sensitive plant, IV is empty vector transformed plant, S1, S2, and S3 are plants transformed with *Alfin1* sense construct and regenerated from transformed callus. #9 is a salt-tolerant control plant. Each blot was hybridized sequentially with the following probes: *Alfin1*, large EcoRI fragment (FIG. 1 (SEQ ID NO: 2); *MsPRP2*, the carboxyterminal and 3'untranslated region fragment; *Msc27*, fragment of a constitutively expressed alfalfa gene to monitor for loading of each lane. Each lane contained 10µg of total RNA. These results demonstrate that increased expression of *Alfin1* led to increased levels of mRNA accumulation from the endogenous *MsPRP2* gene, consistent with *Alfin1* role in *MsPRP2* transcriptional activation. However, this transcriptional activation was root specific, since leaves from the same transgenic plants showed increased *Alfin1* mRNA levels without a concomitant increase in *MsPRP2* transcripts, implying an interaction between *Alfin1* and other gene product(s) present in the root for *MsPRP2* transcriptional activation. Because *Alfin1* contains a very acidic domain as shown in FIG. 1B, just upstream from the postulated zinc finger region, *Alfin1* could interact also with additional factors in binding to DNA. Interestingly, the *MsPRP2* promoter sequence shown in FIG. 3 (SEQ ID NO: 1) contains numerous myc and myb recognition sites, several of which lie in close proximity to the *Alfin1* binding sites, suggesting the possibility of interactions with these transcription factors, similar to those already shown for myc and myb in Arabidopsis (Abe et al., 1997, supra).

On page 19, please amend the following paragraphs:

...specific regulation of *Alfin1* function seems to mostly override this potential problem. Thus accurate assessment of enhanced biotic and abiotic resistance of the *Alfin1* overexpressing transgenics may be even improved by construction of new transgenics in which *Alfin1*

expression will be more tightly under the control of a root specific promoter. Such a promoter, which is the *MsPRP2* promoter shown in FIG. 3 (SEQ ID NO: 1) (has been cloned), for construction of root specific *Alfin1* transgene and to direct additional *Alfin1* expression to roots. Essentially, the 35S promoter for the sense and antisense constructs shown in FIG. 4 is replaced with the 1552 bp promoter of *MsPRP2* and the transformation repeated as before. Since this promoter also binds Alfin1 protein as demonstrated by our current tests, it is believed that these root specific *Alfin1* transgenics will perform even better than the *Alfin1* sense transgenics under the 35S promoter shown by the current results obtained.

The *Alfin1* transgene can be under the control of the CaMV 35S promoter as described. In addition the *Alfin1* transgene can be placed under the control of the full or partial 1500 bp *MsPRP2* promoter FIG 3 (SEQ ID NO: 1) (Bastola, Pethe, and Winicov, 1998, supra) using appropriate restriction sites in the promoter region and *Alfin1* sense construct described in FIG. 4 to construct a new *Alfin1* expression vector for creation of transgenic plants overexpressing the Alfin1 protein.

Changes to the Drawings

As requested in the Office Action, FIGS. 1, 2 and 3 containing DNA sequences have been amended to add the sequence listing numbers.

Attached are marked-up copies of the figures 1A and 3, which will be finalized as soon as one claim is allowed.

Please amend the following lines of FIG. 2:

DNA Sequences that bind Alfin *in vitro*

<u>Clone</u>	<u>Sequence</u>	
	<u>5'primer</u>	<u>Insert</u>
6	5'GACG-GCTGGGGGAAAGTGAGCGGTGGCCC3' (SEQ ID NO: 3)	
7	GACG-CAAAGGGGGTGGGGACGGCGCTTTT (SEQ ID NO: 4)	
18	GACG-CAAAGGGGGTGGGGACGGCGCTTTT (SEQ ID NO: 5)	
15	GACG-GGTAGGGTGTGGGGGGTGTTTTATT (SEQ ID NO: 6)	
16	GACG-GGGATAGGTGAGGTGGAGGGACAAT (SEQ ID NO: 7)	
22	GACG-GCAGAAGGGAGAAACGTGGAGAATC (SEQ ID NO: 8)	
25	GACG-GCGGGAAGGAGTGTGGTAGAGAGCC (SEQ ID NO: 9)	
21	GACG-AAGGAAGGACGGCAGCGTGTTC (SEQ ID NO: 10)	
5	GACG-AAAANTTANANGTGTAGGTGGGACT (SEQ ID NO: 11)	